

1360 Disulfide bonding patterns between β -lactoglobulin and κ -casein in a heated and spray-dried milk-model. A. Bienvenue^{*1}, C.S. Norris², M.J. Boland², L.K. Creamer², and R. Jimenez-Flores¹, ¹DPTC, California Polytechnic State University, San Luis Obispo, CA, ²New Zealand Dairy Research Institute, Palmerston North, New Zealand.

The heat treatment used during the manufacturing of milk powder causes protein interactions that define functionality (Singh, H. & Creamer, L.K. 1991). Moreover, the genetic variant of β -lactoglobulin (β -LG) in homozygous milk influences dramatically its properties during heat processing (Hill, J.P. et al. 1998, US Patent 5,850,804). To better understand the effects of processing on the functionality of milk powder we characterized at the molecular level the protein-protein interactions after heating and drying. The objective of the project was to determine the position of the heat-induced disulfide interchange between β -LG and κ -casein (κ -CN). The powder sample was produced in the pilot plant of the Cal Poly DPTC in San Luis Obispo, CA. A simplified milk system was created by mixing affinity-purified β -LG (β -LG genetic variant AB) to casein micelles obtained by filtration of raw milk (κ -CN variant AA; β -LG variant BB) through a 0.1mm pore size ceramic membrane. The mixture was heated at 90°C for 15 minutes and spray dried. The high molecular weight molecules were segregated by size exclusion chromatography (Rasmussen, L.K. & Petersen, T.E., 1991), identified by SDS-PAGE, and hydrolyzed by trypsin. The native and disulfide-bond-reduced hydrolysates were analyzed by HPLC-MS at the NZDRI Palmerston North, NZ. Our SDS-PAGE analysis shows that we isolated a disulfide-linked protein polymer that contained predominantly κ -CN and β -LG. By comparing our mass spectroscopy results to tryptic digest data banks; we identified 42 peptide fragments including 11 disulfide-linked peptides. We identified three different types of disulfide links: 1) The expected (Rasmussen et al. 1992) intermolecular bridges between two κ -CN molecules connected κ -CN Cys11 to κ -CN Cys11 and κ -CN Cys88 to κ -CN Cys11. 2) The heat induced association of two β -LG linked β -LG AA Cys66 to β -LG BB Cys106/119/121 and β -LG Cys160 to β -LG BB Cys106/119/121. 3) The heat-induced covalent bonding between β -LG and κ -CN involving κ -CN Cys88 to β -LG Cys66, κ -CN Cys11 to β -LG Cys160, and κ -CN Cys11 to β -LG BB Cys106/119/121. These peptides aid in the elucidation of protein interactions in dried milk.

Key Words: Heat-interactions, Disulfide bonds, Milk Powder